Response of *Glyptapanteles militaris* (Walsh) (Hymenoptera: Braconidae), a Larval Parasitoid of the Armyworm, *Mythimna unipuncta* (Haworth) (Lepidoptera: Noctuidae), to Different Temperatures

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Abstract.—The effect of four different temperatures (15, 20 25 and 30°C), on biological parameters of the Azorean population of *Glyptapanteles militaris* (Walsh) was studied, using *Mythimna unipuncta* (Haworth) as the host. Thirteen biological parameters of the host-parasitoid interaction were analysed: percentage of hosts that died without producing parasitoids; percentage of larvae parasitized from which parasitoids emerged; percentage of hosts surviving to pupate after the parasitoid's sting; egg-larval development time; pupal period; total developmental time; adult longevity; total number of larvae per host; number of larval parasitoids that fail to emerge from each host; mean number of parasitoids that emerged from host larva but failed to spin a cocoon; mean number of cocoons per host; parasitoid sex-ratio; and finally emergence rate of adult parasitoid progeny. The percentage of hosts that died without producing parasitoids increased with increasing temperature. Developmental times significantly decreased with increasing temperature. The mean number of cocoons per host; mean number of parasitoids that emerged from each host larva but failed to spin a cocoon; and total number of larvae per host were higher when the temperature was lower. Parasitoid sex ratio and emergence rate of adult progeny were not affected by the temperatures tested.

Mythimna unipuncta (Haworth) is the most important pest in Azorean pastures. Serious population explosions that require the use of pesticides often occur (Tavares 1992). *Glyptapanteles militaris* (Walsh) is a larval parasitoid of the armyworm in all islands of the Archipelago (Oliveira 1996). It is desirable to increase the natural population of *G. militaris* by field releases of wasps produced in the laboratory, during the first generation of *M. unipuncta*.

One of the most important abiotic factors that affect insects is temperature. In parasitoids this can influence development, fecundity, mortality, sex ratio, coloration and other characteristics in various species (Kaya and Tanada 1969; Yu and Luck 1988; Klein 1988; Lysyk and Nealis 1988; Spivac et al. 1992).

Temperature increases, within a favour-able range, will speed up insect metabolism and consequently increase the rate of development. Each species and each stage in the life history may develop at its own rate (Sedlacek et al. 1990, Spivac et al. 1992, Gullan and Cranston 1994).

A previous study of the effect of two different temperatures on the biological parameters of *G. militaris* was performed by Oliveira (1991, 1992). In the present study, we analyse the effect of two extreme temperatures (15 and 30°C) and two intermediate temperatures (20 and 25°C), on some biological parameters of the Azorean population of *G. militaris* using *M. unipuncta* as host.

MATERIAL AND METHODS

Glyptapanteles militaris used in this experiment emerged from naturally parasit-

ized *M. unipuncta* larvae, collected in pastures of São Miguel island. Groups of one hundred cocoons were placed in 400 ml glass vials until adult females were removed for experiments. Adults were supplied with honey solution (10%). We used as hosts *M. unipuncta* larvae from laboratory cultures, established from eggs laid by field-collected females.

On the third day after adult parasitoid emergence, one isolated female wasp was allowed to parasitize one isolated third instar larva of *M. unipuncta*. After the first sting, the host was removed from the parasitoid and individually kept, until emergence of the parasitoids, in a plastic container (4.5 × 3cm). Each host larva was supplied with a small piece (1cm^3) of artificial diet every two days as described by Poitout and Bues (1970) and modified by Oliveira (1991). After parasitization, each group of fifty parasitized host larvae were kept at a different temperature (15±0.5°C, 20±0.5°C, 25±0.5°C and 30±0.5°C), under 75±0.5% R.H. and 16:8 [L:D] photoperiod.

After larval parasitoid emergence and construction of the cocoons, each group was maintained in a plastic container (4.5 × 3cm) with a hole covered by nylon tissue. Emerged adults were kept in the same conditions and were supplied with honey solution (10%) until their death.

Thirteen biological parameters of the host-parasitoid interaction were analysed: percentage of larvae parasitized from which parasitoids emerged; percentage of dead hosts; percentage of hosts surviving to pupate after the parasitoid's sting; egg-larval development time; pupal period; total developmental time; adult longevity; total number of larvae per host; number of larval parasitoids that fail to emerge from each host; mean number of parasitoids that emerged from host larva but failed to spin a cocoon; mean number of cocoons per host; parasitoid sex-ratio (percentage of females); and finally emergence rate of adult progeny.

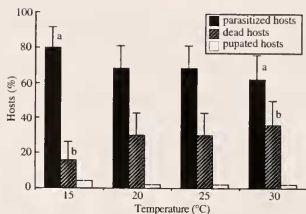The first three parameters were ana-

Fig. 1. Percentages of pupated, dead and parasitized larvae of *Mythimna unipuncta*, at four different temperatures (±standard error). A test for multiple comparison of proportions was used. Each column that is followed by a different letter is significantly different (p < 0.05).

lysed by a test for multiple comparisons of proportions (Zar 1996), and the others parameters were analysed by non-parametric "Kruskal-Wallis" and Multiple Comparison tests (p < 0.05) (Scherrer 1984) to compare the results obtained from different temperatures. To compare the effect of temperature on all studied parameters, a discriminant factorial analysis (Thioulouse 1989), was performed.

RESULTS AND DISCUSSION

Parasitized larvae of *M. unipuncta* were able to develop normally at the four different temperatures, with high percentages of hosts that were successfully parasitized and producing parasite progeny (Fig. 1). However the percentage of parasitized larvae from which parasitoids emerged differed significantly between 15 and 30°C (t = 1.983, p < 0.05), achieving maximum values at 15°C.

The percentage of hosts that died before emergence of the parasitoids increased with temperature. It was least at 15°C, intermediete and similar at 20 and 25°C, and most at 30°C (Fig. 1). A significant difference was observed for the percentage of dead hosts at 15 and 30°C (t = 2.280, p < 0.05). The percentage of surviving hosts after they had been stung by the parasit-

Table 1. Mean (±standard deviation) days of egg-larval development time in *Mythimna unipuncta* (D1), pupal period (D2), and the total developmental time (D1 + D2) of *Glyptapanteles militaris*, at four different temperatures.

Temperature °C	N	D1 $\bar{x} \pm sd$	N	D2 $\bar{x} \pm sd$	N	D1 + D2 $\bar{x} \pm sd$
15	40	37.50 ± 4.90a	40	18.25 ± 1.89a	40	55.75 ± 5.87a
20	34	19.00 ± 2.89b	33	07.27 ± 1.15b	33	26.18 ± 3.07b
25	34	13.56 ± 2.14c	31	05.84 ± 0.69c	31	19.36 ± 2.32c
30	31	11.90 ± 2.01c	20	05.45 ± 1.47c	20	17.15 ± 2.01c
H value		118.495		96.684		108.677
P value		<0.0001		<0.0001		<0.0001

Kruskal-Wallis H and P values and Multiple Comparisons test. Means in each column that are followed by a different letter are significantly different (p < 0.05).

oid was very low (2–4 %) and therefore they were not statistically analysed.

The relation between temperature and rate of development of poikilotherms is an important aspect of ecological studies and basic to the development of pest management strategies (Lysyk and Nealis 1988). According to several authors, the period between parasitization and parasitoid emergence decreases with increasing temperature (Nealis and Fraser 1988; Gould and Elkinton 1990; Allen and Keller 1991; Tillman and Powell 1991). A similar result was obtained in this study with *G. militaris* and *M. unipuncta*. We divided the total development period in two parts: egg-larval development time, and pupal development (strictly, duration of the cocooned stages). We found similar results for both periods (Table 1), and significant differences were observed between the different temperatures with only one exception (25 and 30°C).

Temperature significantly affected the longevity of adult *G. militaris* obtained in this study except between 20 and 25°C. A similar result was obtained by Allen and Keller (1991) in a study of *Cotesia urabae* Austin and Allen reared from *Uraba lugens* Walker. The maximum longevity of *G. militaris* was obtained at 15°C (8.1 days) and the minimum was at 30°C (2.3 days). At the intermediate two temperatures we obtained 2.9 days. Comparing these values with the 16 days of maximum longev-

ity previously observed by Oliveira (1996), we conclude that our results are very low and they may be due to abiotic conditions, such as an insufficient level of ventilation in climatic chambers.

The temperature established during the development time of *G. militaris* can affect the mean number of cocoons found per host, the mean number of parasitoids that emerged from the host larva but failed to spin a cocoon, and the total number of discernable parasitoid larvae. The mean number of cocoons per host decreased with increasing temperature. A significant difference was observed between 30°C and 15 and 20°C (Table 2). Similar results were reported by Oliveira Filho and Foerster (1986) with *"Apanteles" muesebecki* Blanchard parasitising *Pseudaletia sequax* Franclemont. The mean number of parasitoid larvae that emerged from the host but then failed to spin a cocoon was relatively small in each case; the differences found (Table 2), are, though statistically significant, hard to explain in view of the non-linearity of the result and they may not really be informative. The number of parasitoid larvae that failed to leave the host larva was similar at the four temperatures tested (Table 2). The total number of larvae per host decreased with increasing temperature (Table 2). A significant difference was found between the result obtained at 30°C and the other three temperatures.

Table 2. Mean (±standard deviation) number of *Glyptapanteles militaris* cocoons, the number of larvae that failed to spin a cocoon, and that failed to emerge from the host of *Mythimna unipuncta*, at four different temperatures.

Temperature °C	N	N° cocoons/host $\bar{x} \pm sd$	Par. larvae not pupated $\bar{x} \pm sd$	Par. larvae not emerged $\bar{x} \pm sd$	Total larvae $\bar{x} \pm sd$
15	40	55.40 ± 38.24ab	8.50 ± 13.06ab	10.00 ± 16.46a	73.95 ± 31.43a
20	34	50.41 ± 28.71b	2.50 ± 04.86a	8.91 ± 14.83a	61.82 ± 29.25a
25	34	35.12 ± 27.81bc	8.94 ± 15.55ab	12.24 ± 21.84a	56.29 ± 29.21a
30	31	19.42 ± 16.29c	5.55 ± 04.25b	9.19 ± 12.33a	34.16 ± 21.49b
H value		27.36	12.84	3.20	30.19
P value		<0.0001	0.0050	0.3616	<0.0001

Kruskal-Wallis H and P values and Multiple Comparisons test. Means in each column that are followed by a different letter are significantly different (p < 0.05).

The sex ratios (percentage of females) were lower than 19%, in all cases (Table 3). No significant differences as a function of temperature were found. Similar results were obtained by Kolodny-Hirsch (1988) in his study of *Cotesia melanoscela* (Ratzburg) and the host *Lymantria dispar* (L.). In all laboratory cultures of the Azorean *G. militaris* population we systematically obtained low sex ratios (Oliveira 1991, 1992, 1996), when compared with the observed sex ratio of natural populations, usually between 48 and 78% (Oliveira 1991, 1996). This is due to a high percentage of the females that oviposit failing to produce female progeny, indicating a high level of unmated females under laboratory conditions. This is an important aspect that will require to be overcome, if we want to do

mass releases of *G. militaris* to control *M. unipuncta*.

The adult emergence rates were not very high, always less than 71%, but no significant differences were observed between temperatures (Table 3). However, an interesting result was observed at 30°C with a high number of adults dying before complete emergence from the cocoons.

To compare the effect of the different temperatures on all parameters a discriminant factorial analysis was performed. This analysis demonstrated: 1) a superposition of the values obtained at 25 and 30°C; 2) a light separation at 20°C; 3) a complete separation of the results at 15°C (Fig. 2). The parameters that had most influence on this separation were the egg-larval development time, the pupal period, adult longevity, and the number of cocoons per host.

Finally, these results confirm that *G. militaris* is very well adapted to the climatic conditions of the Azores, since the parasitoid develops better between 15 and 20°C than at 25 and 30°C, and the temperatures found on the Azores pastures range between 13 and 23°C during the period of highest activity of the parasitoid.

Table 3. *Glyptapanteles militaris* sex-ratio (percentage of females) and adult emergence rates (±standard deviation), at four different temperatures.

Tempera-ture °C	Sex ratio		% Emergence	
	N	$\bar{x} \pm sd$	N	$\bar{x} \pm sd$
15	40	0.19 ± 0.29	40	70.90 ± 21.80
20	33	0.16 ± 0.23	34	63.00 ± 25.60
25	31	0.18 ± 0.25	34	70.30 ± 25.80
30	21	0.13 ± 0.22	31	49.80 ± 40.70
H value		0.595		5.012
P value		0.8976		0.1709

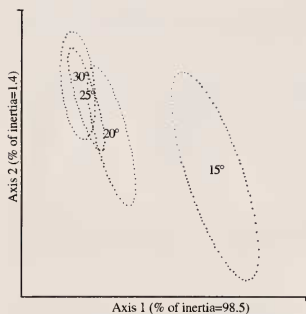Kruskal-Wallis H and P values (p > 0.05).

ACKNOWLEDGMENTS

Fig 2. Discriminant factorial analysis performed with nine biological parameters of *Glyptapanteles militaris*: egg-larval development time, pupal period, adult longevity, mean number of cocoons per host, mean number of parasitoids that emerged from host larva but failed to spin a cocoon, mean number of larval parasitoids that failed to emerge from each host, total number of larvae per host, parasitoid sex ratio, and emergence rate of adult progeny.

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